# MUTAGENIC EFFECTS OF ETHYL METHANESULPHONATE ON THE OAT STEM RUST PATHOGEN (*PUCCINIA GRAMINIS* F. SP. *AVENAE*)

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(Plate vii)

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*Synopsis*

Ethyl methanesulphonate (EMS) induced a high degree of stable variability in the dikaryotic uredial stage of the oat stem rust fungus (*Puccinia graminis* f. sp. *avenae*). Traits affected were uredospore colour, interval between uredial stage and subsequent telial development, virulence and pathogenicity. Mutations for virulence were induced on the oat cultivar Saia. Increases in mutagen concentration, duration of treatment and temperature during treatment significantly increased mutation rates. Recurrent mutagenic treatment increased mutation frequency and in addition produced a wider spectrum of mutants. Potential uses of mutants induced by EMS are indicated.

## Introduction

Ethyl methanesulphonate (EMS) causes mutations in the dikaryotic uredial stage of the oat stem rust fungus, *Puccinia graminis* f. sp. *avenae* Eriks. and Henn. (Baker and Teo, 1966). In their studies aberrant uredospore colour and rapid telial-forming variants were described. The present studies provide estimates of mutation rates for these traits, and describe the effects of mutagen concentration, duration of treatment and temperature during treatment on mutation rates. In addition, alterations in virulence and changes in certain pathogenic traits were demonstrated.

## Materials and Methods

The strains of oat rust used were 2–0, 7–H and 8–H, the cultures of which are designated as BC1, BC2 and BC3, respectively, in the University of Sydney Rust Accession Register. Infection types produced on the oat differential varieties when inoculated with these cultures are shown in Table 1.

Table 1

*Infection types exhibited on seedlings of various oat cultivars (resistance gene(s) in brackets) by three cultures of oat stem rust at 17±2°C*

| Cultivar | Rust culture | | |
|---|---|---|---|
| | BC1 | BC2 | BC3 |
| (*a*) Canadian differentials | | | |
| Richland, *08 (*A*) | 1+ | 1 | 4 |
| Rodney, 0654 (*B*) | 2— | 1— | 1 |
| White Tartar, 05 (*D*) | 2= 2— | 4 | 2— |
| Jostrain, 0617 (*E*) | 3+ | 4 | 4 |
| Eagle²×C.I.4023, 0658 (*F*) | 2= 2— | 2 | 2— |
| C.I.5844·1, 0661 (*H*) | 2= 2— 3c | 3 | 3 |
| (*b*) Supplementary differentials | | | |
| Minnesota Ag.331, 0615 (*A D*) | 1+ | 1+ | 2— |
| Saia, 0589 (Sa) | ; 1 | ; 1 | ; 1 |
| Strain designation | 2†–0‡ | 7–H | 8–H |
| Virulence formula (Green, 1963) | ABDFH/E§ | ABF/DEH | BDF/AEH |

* 0 numbers refer to Sydney University Oat Accession Register.

† Race designation according to Newton and Johnson (1944).

‡ The symbol 0 indicates avirulence on genes *A*, *B*, *D*, *F* and *H*. The symbol H indicates virulence on gene *H*.

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Seedling infection types, recorded 14–16 days after inoculation, were designated in accordance with the scale suggested by Stakman *et al.* (1923).

The oat cultivars employed were Bond and Saia. Bond is a susceptible hexaploid cultivar. Saia (an accession of *Avena strigosa* Schreb., a diploid species) is resistant to most oat stem rust strains in Australia. However, certain field isolates collected in eastern Australia (Luig and Baker, 1973) have proved virulent on this cultivar.

The current experiments were conducted to study, in part, the effect of mutagen concentration, duration of treatment, and temperature during treatment, on mutation rate for certain rust characters. In this regard culture BC2 was treated as follows :

1. With $9{\cdot}8\times10^{-2}$, $14{\cdot}7\times10^{-2}$ and $19{\cdot}6\times10^{-2}$M aqueous solutions of EMS for 2·0 hrs at 25°C. Distilled water was used in the preparation of the solutions. (M.W. of EMS is 124·16 and its density at 20°C was determined to be 1·2175).
2. For 2·0, 3·5 and 5·4 hrs with a $9{\cdot}8\times10^{-2}$M solution at 20°C in separate experiments.
3. At 20, 25 and 30°C for 2·0 hrs with a $9{\cdot}8\times10^{-2}$M solution.

Cultures BC1 and BC3 were also treated with EMS, each with a $14{\cdot}7\times10^{-2}$M solution for 2·0 hrs at 20°C. Finally, the effect of treating an EMS-induced grey-brown uredospore colour mutant of culture BC2 with $12{\cdot}5\times10^{-2}$M EMS solution for 2·0 hrs at 22°C was studied.

In recurrent mutagenic treatments cultures BC1 and BC2 were used. The former was treated for four and the latter for five successive uredial generations with $14{\cdot}7\times10^{-2}$M EMS for 2·0 hrs at room temperature. Cultures BC1 and BC2 were also treated for two and three consecutive uredial generations, respectively, with $15{\cdot}7\times10^{-2}$M EMS for 2·0 hrs at room temperatures in attempts to induce mutations for virulence on the cultivar Saia.

Uredospores were treated with solutions of EMS (100 mls per 0·2g of spores) in a stoppered flask shaken with a Griffin flask shaker. After treatment, the spores were rapidly filtered in a Buchner filtering apparatus. After washing, filtering was continued to remove as much water as possible from the spore mass. A sample was retained for assessment of spore viability. The remainder was dispersed in " Mobil Sol 100 " (Rowell and Olien, 1957) and the suspension immediately sprayed with a " Desaga Spray Can " atomiser onto primary leaves of 1,500–2,000 seedlings of Bond. However, in experiments designed to induce mutations for virulence, 1,500–2,000 seedlings of Saia and 300 seedlings of Bond (to provide inoculum for the next recurrent mutagenic treatment) were inoculated simultaneously. A control experiment set up in each case comprised 500–600 seedlings of the appropriate cultivar inoculated with untreated spore samples. Inoculated plants were placed in misting chambers for 12–24 hrs and then transferred to well-lit glasshouse benches.

*Assessment of Spore Germination*

Samples of mutagen-treated and untreated spores were spread lightly and evenly on 2% water-agar in three petri dishes and placed in an incubator at 18–20°C for 24 hours. After this period, germination of at least 500 spores per dish was examined. The percentage spore germination was determined after totalling the results for each dish.

Spores were classified as inviable if no germination was shown or if initial germination occurred but germ tubes failed to continue growth. In the latter the germ tube barely appeared through a pore in the wall after 24 hours whereas germ tubes from viable spores showed extensive hyphal development after the same period.

*Assessment of Mutation Rate*

All leaves were examined and the number of mutants recorded. The total number of pustules appearing on all leaves was then counted. In certain experiments, where indicated, the total number of pustules was assessed by sampling. In these instances, the number of pustules on seedlings in ten randomly selected pots (approximately 35 seedlings per pot) was counted and the approximate total number of pustules in the experiment estimated on the basis of such sampling. This method of estimation was less reliable as the rate of infection varied somewhat from pot to pot. However, slight inaccuracies in the estimation of the total number of pustules by sampling were shown to have no significant effect on statistical assessment of mutation rate.

## Experimental Results

*Induction of Mutants*

During the course of various experiments spore viability following EMS treatments under similar conditions was found to vary markedly depending on the source and batch of the chemical. For example, spore viability ranged from 0–96% of controls (which showed 85–92% viability) after treatment with $9{\cdot}8\times10^{-2}$M solutions for 2·0 hrs at 20°C in different experiments. Apparently EMS may contain impurities, such as mercapto ethanol, which are fungicidal, thus presumably accounting for reduced spore viability, the variation in which may have been due to relative amounts of impurities.

It was not possible to obtain EMS free from impurities on all occasions. Consequently, the use of EMS from sources which did not result in undue lethality offered the only practical solution to this problem. In experiments where strict comparisons were necessary, as in studies concerned with the effect of treatment variables, EMS relatively free from impurities and from the same bottle was used in all treatments.

Within the range of reduction in spore viability tolerated there was no evidence that impurities affected either the mutation rate or the type of mutants.

The influence of treatment conditions on spore viability and mutation rate in culture BC2 is recorded in Table 2. Spore viability decreased with increases in the mutagen concentration, duration of treatment and temperature during treatment. Conversely, the total mutation rate (which was based on both aberrant colour mutants and rapid telial-forming variants) significantly increased with increases in mutagen concentration, duration of treatment and temperature during treatment. A similar result was obtained when the rate of colour mutants alone was considered. In the case of rapid telial-forming variants increases in rate were significant only in instances where the 95% limits of expectation (calculated by the method of Stevens (1972)) did not overlap.

Two alternatives for determining mutation rates can be considered. The mutation rate can be estimated in terms of the total number of spores treated or in relation to the total number of spores which germinated and produced pustules. For practical considerations the latter estimate was used in these studies.

As with culture BC2, yellow and orange colour mutants and rapid telial-forming variants were readily induced in cultures BC1 and BC3 (Table 3). Mutation rates for these traits in the three strains did not differ significantly. No mutants were observed in the control experiments.

The results of recurrent mutagenic treatments of cultures BC1 and BC2 are shown in Table 4. With each successive cycle statistically significant increases in total mutation rate occurred. Moreover, a wider range of aberrant uredospore colour types was obtained. Grey-brown colour mutants, which were not obtained following single mutagenic treatment except on one occasion at the highest

TABLE 2

*Effect of concentration, duration and temperature of EMS treatments on uredospore viability and mutation rate in culture BC2 of oat stem rust*

| Treatment | | Spore viability (% control) | Total number pustules | Uredospore colour mutants: Yellow and orange no. | Grey-brown no. | Total no. | Mutation rate and 95% limits ($\times 10^{-3}$) O* | L | U | Rapid telial-forming variants: No. | Mutation rate and 95% limits ($\times 10^{-4}$) O | L | U | Total mutants: No. | Mutation rate and 95% limits ($\times 10^{-3}$) O | L | U |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2.0 hrs, 25°C | $9{\cdot}8\times10^{-2}$M | 82·3 | 11,724 | 60 | — | 60 | 5·12 | 3·91 | 6·58 | 2 | 1·71 | 0·22 | 4·24 | 62 | 5·29 | 4·06 | 6·78 |
| | $14{\cdot}7\times10^{-2}$M | 52·1 | 5,592 | 57 | — | 57 | 10·19 | 7·73 | 13·19 | 5 | 8·94 | 2·90 | 20·87 | 62 | 11·09 | 8·51 | 14·21 |
| | $19{\cdot}6\times10^{-2}$M | 35·9 | 31,949 | 556 | 3 | 559 | 17·50 | 16·09 | 19·00 | 42 | 13·15 | 9·48 | 17·78 | 601 | 18·81 | 17·35 | 20·36 |
| $9.8\times10^{-2}$M, 20°C | 2·0 hrs | 94·0 | 14,092 | 28 | — | 28 | 1·99 | 1·32 | 2·81 | 2 | 1·42 | 0·18 | 3·53 | 30 | 2·13 | 1·44 | 3·04 |
| | 3·5 hrs | 85·4 | 12,319 | 65 | — | 65 | 5·28 | 4·07 | 6·72 | 5 | 4·06 | 1·31 | 9·47 | 70 | 5·68 | 4·43 | 7·18 |
| | 5·4 hrs | 58·2 | 16,964 | 187 | — | 187 | 11·02 | 9·51 | 12·12 | 9 | 5·30 | 2·43 | 10·07 | 196 | 11·55 | 10·00 | 13·28 |
| $9.8\times10^{-2}$M, 2.0 hrs | 20°C | 96·0 | 10,413 | 22 | — | 22 | 2·11 | 1·32 | 3·20 | 2 | 1·92 | 0·23 | 6·93 | 24 | 2·30 | 1·49 | 3·43 |
| | 25°C | 79·6 | 12,741 | 72 | — | 72 | 5·65 | 4·40 | 7·13 | 4 | 3·14 | 0·85 | 8·04 | 76 | 5·96 | 4·70 | 7·46 |
| | 30°C | 19·7 | 10,373 | 126 | — | 126 | 12·15 | 10·13 | 14·45 | 11 | 10·60 | 5·29 | 18·97 | 137 | 13·21 | 11·09 | 15·61 |

* O = Observed ; L = Lower limit ; U = Upper limit.

TABLE 3

*Comparative effects of similar mutagenic treatments on uredospore viability and mutation rates in three cultures of oat stem rust*

| Treatment | Rust culture | Spore viability (% control) | Total number pustules | Yellow and orange uredospore colour mutants: No. | Mutation rate and 95% limits ($\times 10^{-3}$) O* | L | U | Rapid telial-forming variants: No. | Mutation rate and 95% limits ($\times 10^{-4}$) O | L | U | Total mutants: No. | Mutation rate and 95% limits ($\times 10^{-3}$) O | L | U |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $14{\cdot}7\times10^{-2}$M EMS, 2·0 hrs 20±1°C | BC1 | 50·2 | 12,613 | 38 | 3·01 | 2·13 | 4·13 | 9 | 7·13 | 3·27 | 13·54 | 47 | 3·73 | 2·74 | 4·95 |
| | BC2 | 47·5 | 15,050 | 63 | 4·19 | 3·22 | 5·35 | 8 | 5·31 | 2·29 | 10·47 | 71 | 4·72 | 3·69 | 5·95 |
| | BC3 | 51·6 | 6,843 | 22 | 3·21 | 2·02 | 4·86 | 6 | 8·77 | 3·21 | 19·08 | 28 | 4·09 | 2·72 | 4·74 |

* O = Observed ; L = Lower limit ; U = Upper limit.

TAB
*Effect of recurrent mutagenic treatments on mutat*

| Rust culture | Treatment | | Recurrent cycle number | Spore viability (% control) | Total number pustules | Uredospore col | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Yellow and orange no. | Grey-brown no. | Tota no. |
| BC1 | $14{\cdot}7\times10^{-2}$M, 2·0 hrs | 20±1°C | Initial | 50·2 | 12,163 | 38 | — | 38 |
| | | 22±1°C | 1 | 38·2 | 11,353 | 90 | — | 90 |
| | | 22±1°C | 2 | 45·8 | 8,917 | 157 | 3 | 160 |
| | | 19±1°C | 3 | 57·1 | 7,688 | 244 | 4 | 248 |
| BC2 | $14{\cdot}7\times10^{-2}$M, 2·0 hrs | 20±1°C | Initial | 47·5 | 15,050 | 63 | — | 63 |
| | | 21±1°C | 1 | 43·4 | 11,439 | 118 | 2 | 120 |
| | | 19±1°C | 2 | 59·6 | 7,302 | 109 | 5 | 114 |
| | | 22±1°C | 3 | 37·7 | 10,725 | 329 | 3 | 333 |
| | | 19±1°C | 4 | 53·4 | 8,120 | 416 | 7 | 423 |

* O = Observed ; L = Lower limit ; U = Upper limit.

EMS concentration (Table 2), were readily induced following recurrent mutagenic treatments. Mutants possessing a markedly darker spore colour than normal were also found. Certain variants categorised in Table 4 as showing rapid telial formation were also characterised by aberrant uredospore colour.

In all of the above, single or recurrent mutagenic treatments mutations for pathogenicity were observed. In the current context, usage of the terms "pathogenicity" and "virulence" follows that adopted by Watson (1970). Pathogenicity is a general term and is used in connection with such characters observed on the host as pustule size, volume of spores produced and the rate of growth of the pathogen. If a strain is virulent on a host, the gene or genes for resistance in the host are matched in the fungus by corresponding genes for virulence.

Pathogenic mutants exhibited either small uredopustule size or delayed pustule growth and development. Pathogenic mutants exhibiting subepidermal uredial formation were also found following recurrent mutagenic treatments. Certain aberrant colour mutants were characterised by one or more such pathogenic traits.

No mutants virulent on Saia were induced following initial EMS treatments. However, virulent mutants were induced in cultures BC1 and BC2 after one and two recurrent mutagenic treatments, respectively (Table 5).

TABLE 5
*Effect of recurrent EMS treatments on mutation rates for virulence on Saia BC1 and BC2 of oat stem rust*

| Rust culture | Pertinent virulence gene* | Treatment | Recurrent cycle number | Total number pustules | Virulence mutants ($v_{Sa}$) | |
|---|---|---|---|---|---|---|
| | | | | | Number | Rate ($\times10^{-4}$) |
| BC1 | $V_{Sa}$ | $15{\cdot}7\times10^{-2}$M, 2 hrs, 21±1°C | Initial | 20,000† | — | |
| | | 2 hrs, 21±1°C | 1 | 25,600† | 4 | 1·56 |
| BC2 | $V_{Sa}$ | $15{\cdot}7\times10^{-2}$M, 2 hrs, 21±1°C | Initial | 22,700† | — | |
| | | 2 hrs, 21±1°C | 1 | 23,400† | — | |
| | | 2 hrs, 21±1°C | 2 | 24,850† | 2 | 0·80 |

$V_{Sa}$ and $v_{Sa}$ indicate avirulence and virulence on Saia, respectively.
* A single dominant gene was considered to govern resistance (see Discussion).
† Estimated by sampling procedure.

*in cultures BC1 and BC2 of oat stem rust*

| ants | Rapid telial-forming variants with uredial colour | | | | | Total mutants | | | |
|---|---|---|---|---|---|---|---|---|---|
| lutation rate ($\times 10^{-3}$) | Normal no. | Yellow and orange no. | Grey-brown no. | Total no. | Mutation rate ($\times 10^{-4}$) | No. | Mutation rate and 95% limits ($\times 10^{-3}$) O* | L | U |
| 3·01 | 9 | | | 9 | 7·13 | 47 | 3·73 | 2·74 | 4·95 |
| 7·93 | 12 | 1 | | 13 | 11·45 | 103 | 9·07 | 7·41 | 10·99 |
| 17·94 | 25 | | | 25 | 28·04 | 185 | 20·75 | 17·78 | 23·93 |
| 32·26 | 19 | 1 | 1 | 21 | 27·31 | 269 | 34·99 | 31·01 | 39·36 |
| 4·19 | 8 | | | 8 | 5·31 | 71 | 4·72 | 3·69 | 5·95 |
| 10·49 | 14 | | | 14 | 12·24 | 134 | 11·71 | 9·83 | 13·86 |
| 15·61 | 16 | 1 | | 17 | 23·28 | 131 | 17·94 | 15·03 | 21·26 |
| 30·96 | 43 | 3 | | 46 | 42·89 | 378 | 35·24 | 31·84 | 38·92 |
| 52·09 | 38 | 4 | | 42 | 51·72 | 465 | 57·70 | 52·33 | 62·56 |

Subjecting an EMS-induced grey-brown colour mutant to mutagenic treatment yielded new variants. Of a total of approximately 20,000 pustules (estimated by sampling procedures) examined, eight white uredospore mutants were found.

The mutants, excepting those characterised by induced virulence on Saia, will be described in a subsequent paper.

*Mutants Virulent on Cultivar Saia*

The infection types produced by two virulent mutants of culture BC2 compared with the original culture are shown in Plate VII.

The four mutants virulent on Saia induced from culture BC1 exhibited identical infection types when tested on differential sets. The two mutants virulent on Saia induced from culture BC2 also produced identical infection types on differential sets. Characteristic infection types produced on differential sets by one representative mutant virulent on Saia induced from each of cultures BC1 and BC2 are shown in Table 6.

TABLE 6

*Infection types exhibited on seedlings of various oat cultivars (resistance gene(s) in brackets) at 17 ±2°C by mutants of oat stem rust with induced virulence*

| | Cultivar | Mutant rust culture BC1–a | BC2–a |
|---|---|---|---|
| (*a*) | Canadian differentials | | |
| | Richland, 08 (*A*) | 1— 1 | 1— 1+ |
| | Rodney, 0654 (*B*) | ; 1 | ; 1— |
| | White Tartar, 05 (*D*) | 1+ 2= | 3 3+ |
| | Jostrain, 0617 (*E*) | 3c | 3 3+ |
| | Eagle[2] × C.I.4023, 0658 (*F*) | 2= 2— | 1+ 2 |
| | C.I.5844·1, 0661 (*H*) | 1+ 2 3c | 3 3+ |
| (*b*) | Supplementary differentials | | |
| | Minnesota Ag. 331, 0615 (*A D*) | ; 1+ | 1 1+ |
| | Saia, 0589 (Sa) | 3c | 3 3+ |
| | Strain designation | 2–0–1* | 7–H–1 |

* Indicates virulence on Saia.

Except for virulence on Saia, mutants produced no changes in infection types when tested on genotypes constituting the differential set. However, mutants of culture BC1 gave " 3c " infection types on the universally susceptible cultivars Algerian, Bond, Fulghum, Victoria and Belar compared with " 4 " infection types produced on these cultivars by the original culture. Likewise, on these susceptible cultivars mutants of culture BC2 produced infection types (" 3 3+" with some chlorosis) similar to those exhibited on Saia. These were slightly but distinctly lower than the " 4 " infection types produced on these cultivars by the original cultures. Further, mutants showed 2–3 days' longer incubation and developmental periods and a general reduction in sporulation vigour compared with the respective original strains. These observations suggest that associated changes in pathogenicity were implicated in the production of these mutants.

## Discussion

Ethyl methanesulphonate proved highly mutagenic on *Puccinia graminis avenae*. Variability induced in the dikaryotic uredial stage involved traits differing as widely as uredospore colour, interval between uredial stage and subsequent telial development, virulence and pathogenicity.

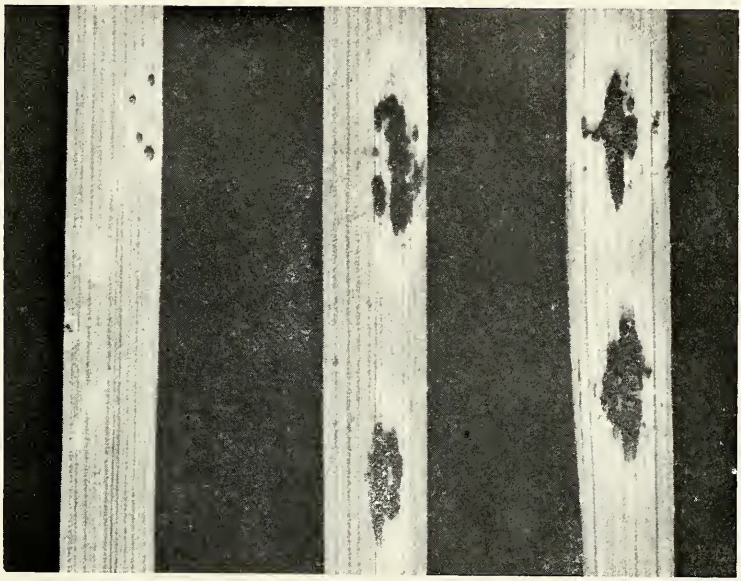
Genetic rather than temporary phenotypic changes were involved in the variability induced. Mutants remained stable over several generations of subculturing. Contingent upon proof from inheritance studies, the changes induced are believed to be due to recessive chromosomal gene mutations.

Mutants virulent on Saia differed from the original strains only by high infection types produced on this cultivar. The number of genes conditioning resistance in Saia is not definitely known. Murphy *et al.* (1958) concluded that the same two independent dominant genes in *Avena strigosa* accession C.D.3820 (which they considered agronomically identical with Saia) conditioned resistance to each of four races. On the other hand, Dyck (1966) found that the same single dominant gene in several accessions of *A. strigosa* governed resistance to each of a wide range of races. If Dyck's conclusions are accepted on the basis of the gene-for-gene hypothesis (Flor, 1956) mutations for virulence on Saia would involve a single locus and be recessive since avirulence is usually dominant.

Except for mutants virulent on Saia, no alterations in virulence were shown by the variants examined. Although certain aberrant colour mutants exhibited rapid telial formation or one or more pathogenic changes, absence of invariable association between the various traits indicated that independent mutations were involved. However, changes in incubation and developmental periods associated with a change in spore colour may have been due to pleiotropic gene effects.

The variants induced were, therefore, intrinsically similar to those reported to arise from spontaneous mutation or genetic recombination, although the rate of, and range in variability induced was greater than that ascribed to spontaneous mutation. This does not imply that such types would not occur naturally at a low frequency. However, most abnormal variants would obviously have low survival ability under natural conditions and would not be expected to persist.

Mutations for virulence were less readily induced than variation in other traits. Mutations for uredospore colour, particularly those affecting loss of spore wall pigment formation resulting in yellow or orange uredosori, occurred at the highest frequency. Increases in mutagen concentration, duration of treatment and temperature during treatment increased significantly mutation rate for colour mutants and rapid telial-forming variants. Recurrent mutagenic treatment also increased mutation frequency and, in addition, produced a wider spectrum of mutants.



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